Please replace the paragraph beginning at page 1, line 27, with the following rewritten paragraph.

In addition to technical aspects, pectins also involve physiological aspects. The citation Cerda, J.J., Trans. Am. Clin. Climatol. Assoc. 99, 203-213 (1987), describes that pectin from grapefruits plays an important role in promoting health in consumers. The citation Matsumoto, T. et al, Int. J. Immunopharmacol. 15, 683-693 (1993), describes that particular fragments of Bupleuran 2IIc, a pectin-like poly-saccharide from the roots of *Bupleurum falcatum*, might be highly important in pharmaceutical terms. The fragments are obtained by reaction with ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15). Ultimately, the citation Voragen, A.G.J., Trends Food Sci. Technol. 9, 328-335 (1998), provides information that non-digestible polysaccharides or oligosaccharides may have a number of health-promoting effects on persons consuming same.

Please replace the paragraph beginning at page 2, line 13, with the following rewritten paragraph.

Producing oligosaccharides for pharmaceutical purposes from pectins using pectinases is known from the citation US-A-5,683,991. The pectinases used therein are mixtures of various enzymes which possibility also include ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15), so that cleavage also is effected in the side chain regions. Moreover, the ester groups are hydrolyzed prior to reacting with the pectinases so that, as a result, comparatively small oligosaccharides (2-4 monomers) are obtained.

Please replace the paragraph beginning at page 2, line 31, with the following rewritten paragraph.

To solve said technical problem, the invention teaches the use of polygalacturonides as additives in goods, said polygalacturonides being obtainable via the following process steps:

1. a pectinous plant material is subjected to a pectin extraction is aqueous solution;
2. the solids are removed from the suspension obtained in step a), consisting of liquid phase including dissolved pectin and solids from the plant material;
3. the pectin is precipitated from the liquid phase obtained in step b);
4. the pectin obtained in step c) is dissolved in an aqueous solution and cleaved with purified ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15);
5. the polygalacturonides obtained in step d) are processed into a polygalacturonide preparation with using an additional separation step and without hydrolyzing ester groups that are present.

Within the range of the invention, it is essential that no hydrolysis of ester groups takes place and that, owing to the use of purified ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15), only bonds in (naturally) non-esterified galacturonic acid monomer units undergo cleavage. As a result, a higher amount of comparatively large oligosaccharides, e.g., with 5-20 monomer units (main and optionally side chains), is obtained. Here, virtually all of the polygalacturonides are

saturated polygalacturonides, they are less reactive compared to the unsaturated polygalacturonides formed by pectin lyase or pectate lyase activity and therefore do not contribute to non-enzymatic browning and formation of hydroxymethylfurfural (HMF) from fructose or glucose. The mixture of various oligosaccharide structures thus obtained has distinct advantages. On the one hand, fragments with largely retained side chains have a supporting effect on the immune system in physiological terms. In addition, the comparatively long oligosaccharides of the invention assume a roughage effect, as is the case with other long-chain polyers as well. Roughage is important in the prophylaxis and therapy of a number of diseases such as constipation, diverticulosis, colon carcinoma, diabetes mellitus, and lipid metabolic diseases. However, the drawbacks of conventional roughage, namely, binding of essential nutrients, will be reduced when using oligosaccharides of the invention ranging from 5 to 20 monomer units. Moreover, by virtue of the intestinal bacterial flora as found in organisms, the polygalacturonides according to the invention are converted relatively readily to short-chain fatty acids such as acetate, butyrate and propionate which in turn have a positive effect on the intestinal flora and on the intestinal pH value. These fatty acids can be utilized energetically by the organism (energy content of the basic oligosaccharides: about 2 kcal/g), serving particularly mucosa blood circulation as well. In addition, the polygalacturonides used according to the invention have an antibacterial and emulsion-stabilizing effect, which is particularly advantageous in food-technological terms. This also enables the use as fat substitutes, e.g., in mayonnaises and the like.

Please replace the paragraph beginning at page 5, line 24, with the following rewritten paragraph.

Preferably, step d) is performed using a pH of from 1.4 to 8.2, more preferably from 3.5 to 5.0. The ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) which is used can be recovered from plants or from microorganisms occurring therein, e.g., cotton/*Aspergillus flavus/Aspergillus parasiticus*, rye/*Claviceps purpurea*, maize/*Cochliobolus carbonum/Fusarium monilifonne*, American walnut/*Cryphonectria parasitica*, tomato/*Fusarium oxysporum/Ralstonia* (Pseudonomas) *solanacearum*, rice/*Rhizoctonia solani*, grass/*Sclerotinia borealis*, sunflower/*Sclerotinia sclerotiorum*, and apple/*Stereum pureum*, carrot/*Ertwinia carotorova* or *Burkholderia* (Pseudomonas) *cepacia*, or from microorganisms genetically engineered so as to produce a well-defined ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15). The latter is preferred for its comparatively easy purification. In addition to well-known genes of the above-exemplified microorganisms, well-known cDNAs or cDNAs from plants themselves which encode well-known enzyme structures are possible for recombinant operations. Examples of well-known plant cDNAs are those from *Arabidopsis thaliana, Persea americanus* and *Prunus persica*. Examples of well-known ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) of plants are those from *Lycopersicon esculentum, Musa acuminata, Gossypium barbadense, Gossypium hirsutum, Cucumus sativus, Phaseolus vulgaris, Citrus limon, Mangifers indica, Cucumis melo, Passiflora edulis, Prunus persica, Pyrus communis, Rubus idaeus*, and *Fragaria ananassa*. It is preferred to use one or more ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) selected from the ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) which can be obtained from organisms of the group consisting

of *Aspergillus carbonarius, Aspergillus niger, Aspergillus oryzae, Aspergillus tubingensis, Aspergillus ustus, Kluyveromyces marxianus, Neurospora crassa, Penicillium frequentans*, and *Saccharomyces cerevisiae SCPP, Lactobacillus casei, Lactobacillus plantarum, Lactococcus lactis, Bacteroides thetaiotaomicron, Piromonas communis, Neocalimastix patriciarum*, or from other microorganisms modified with DNA sequences of the above organisms which encode the ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15). The purification can be effected using e.g. the gel filtration technology which is well-known to those skilled in the art. The amount of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) employed in step d) advantageously ranges from 10 to 1000, preferably from 20 to 400 U/g pectin. Determination of the activity is effected using the following method:

10 mg/l polygalacturonic acid (e.g. Sigma P 7276, according to the product specification valid on March 01, 2000) is dissolved in substrate buffer (2 mM citric acid solution, 1 mM $CaCl_2$) and added with defined quantity of enzyme solution. Incubation is effected for 15 min at 23°C. The reaction then is stopped by adding an equal volume of 4.4 mM 2-hydroxy-3, 5-dinitrobenzoic acid solution, boiling for 5 min, and cooling to 0°C. Eventually, the absorption at 540 nm is detected, and the conversion is determined from these values against a standard curve in the usual manner. One unit is the conversion of 1 µM of galacturonic acid per minute.

Please replace the paragraph beginning at page 7, line 10, with the following rewritten paragraph.

In a particularly preferred embodiment of the invention, the ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) is immobilized by inclusion in enzyme membrane reactors, e.g.

flat membrane or hollow fiber membrane reactors, or binding to a conventionally prepared support material can be effected in an absorptive, ionic chelating, covalent way, or by crosslinking.

The immobilization allows to make sure that the final product is virtually free of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) and, in fact, without an extra separation. In this way, interfering reactions due to otherwise possible entraining of the employed ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) with pectins possibility included in the food is virtually excluded. In particular, this is advantageous because undesirable decomposition of pectins included in the foods might have an unfavorable effect on the consistency of the foods, e.g. the viscosity thereof. Moreover, the ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) consumption is comparatively low.

The reaction with ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) in step d) preferably is effected at 4 to 80°C, more preferably 30 to 70°Cm, for 2 to 300 min, preferably 45 to 150 min. The operations can be performed in a continuous or discontinuous (batchwise) fashion. As to the continuous process, the above-mentioned time period relates to the average residence time in a reaction volume containing ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15).

Please replace the paragraph beginning at page 8, line 10, with the following rewritten paragraph.

Example 1:

Isolation and purification of a ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) from tomatoes.

Tomatoes, 1 kg, are homogenized in 1 liter (1) of water, and the suspension obtained is adjusted to pH 3.0. The solids (cell residues) are removed by centrifugation (10,000 g, 20 min) and washed in water. The pellets are taken up to 50 mM sodium acetate/1.25 mM NaCl (ph 6.0) at 4°C for 1 hour. Proteins are precipitated by means of 70% ammonium sulfate saturation and removed by centrifuging (10,000 g, 20 min). The protein pellet is dissolved in 0.125 M sodium acetate (pH 6.0) and dialyzed against said buffer. The proteins then are separated on a CM Sepharose column in a 2-stage gradient (0.45 M sodium acetate, pH 6.0, and 1.0 M sodium acetate, pH 6.0). The ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) elutes with the first stage.

Example 2:

Preparation and purification of a ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) from genetically engineered microorganisms.

Yeast (*Saccharomyces cerevisiae*) is transformed using an expression plasmid including cDNA of the *Aspergillus niger* ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) gene under yeast ADH1 promoter control.

The plasmid still includes the yeast replication origin and yeast selection marker (e.g. LEU2 gene). The yeast strain obtained is pre-grown in nutrient medium (minimal medium) and cultured in a fermentation process wherein the ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) is discharged into the nutrient medium.

Please replace the paragraph beginning at page 9, line 10, with the following rewritten paragraph.

The clear medium supernatant is transferred on a carboxymethylcellulose cation exchange column and equilibrated with 10 mM sodium acetate (pH 4.0). The proteins are eluted using a linear gradient of from 1 to 1.5 M NaCl in 10 mM sodium acetate (pH 4.0). Fractions including pure ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) elute between 0.6 and 0.75 M NaCl.

Please replace the paragraph beginning at page 9, line 29, with the following rewritten paragraph.

The ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) is eluted in the salt gradient.

Please replace the paragraph beginning at page 10, line 3, with the following rewritten paragraph.

3 g of a particulate carrier, e.g. silicate or glass carrier derivatized with surface-bound NH_2 groups (Solvay Enzymes, Hanover), is suspended in 20 ml of 0.01 M Sørensen phosphate buffer (pH 7.0) and degassed. 0.2 g, i.e. 1000 U of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase

(EC 3.2.1.15) from Example 3 is dissolved in 5 ml buffer, pH 7.0, and added to the carrier suspension. With stirring, the decline of the extinction at 280 nm is monitored for about 30 to 60 min. The solution is removed, followed by washing 5 times with water, and coupling is effected by adding 10 ml of glutaric dialdehyde solution (5%) and stirring for 30 min. Thereafter, this is washed 5 times in water, 1 hour, followed by washing 3 times with buffer (pH 5.0). Finally, this is suspended in 50 ml of buffer, pH 5.0, to obtain covalently immobilized ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15).

Please replace the paragraph beginning at page 10, line 28, with the following rewritten paragraph.

The pectin is precipitated by adding 4 parts by weight of ethanol (96%) per part by weight of solution and separated from the liquid phase by centrifuging (10,000 g for 10 min) at 4°C, optionally with subsequent dialysis. The pectin obtained then is dissolved in a 0.1 M sodium acetate buffer (pH 4.2) and reacted with 300 U/g pectin employed of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) (from *Aspergillus japonicus*, available from the Sigma Company under the product designation P 3304, according to the product specifications valid on March 01, 2000) for 4 hours at 37°C, said reaction being done batchwise. The polygalacturonides obtained are recovered in solid form by evaporating the water.

Please replace the paragraph beginning at page 11, line 10, with the following rewritten paragraph.

Beet pressed chips, 1 kg, from the beet-processing industry are extracted in 15 kg of an acidic aqueous solution (phosphoric acid, pH 1.5) for 1 hour at 90°C. Following cooling to 20°C, the solid phase is removed by hydraulic pressing and optional filtration. Using vacuum evaporation, this is concentrated to 1/10 the volume of the liquid phase. The pectin is precipitated by adding 2 parts by weight of isopropanol per part by weight of concentrate. Following centrifugation (10,000 g, 10 min) and drying (70°C, 1 hour), the enzymatic hydrolysis is accomplished by adding an aqueous solution of 5 g/kg water (adjusted to pH 4.0) of pectin extract with 20 U/g pectin of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) from Example 1 and performing the reaction for 60 min at 60°C. The polygalacturonides obtained are recovered in solid form by evaporating the water.

Please replace the paragraph beginning at page 12, line 10, with the following rewritten paragraph.

500 mg of ~~*endo*-polygalacturonase~~ *endo*-polygalacturonase (EC 3.2.1.15) is dissolved in 15 ml of distilled water. With stirring in an ice bath, 30 ml of ice3-cold acetone is slowly added, followed by addition of 2 ml of a 25% glutaric dialdehyde solution. Thereafter, this is agitated for 60 min at 30°C and subsequently centrifuged. The supernatant is discarded, and the residue is stirred up with 40 ml of distilled water and homogenized using an Ultra Turrax. Following centrifugation and discarding of the supernatant, the residue is washed once more with 40 ml of distilled water. The crosslinked preparation obtained is suspended to make 100 ml.